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Optimization of Variables in Growth Media of Two Coral Endosymbiotic Fungi for Potential Biological Activities using Taguchi Statistical Design

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ABSTRACT

Taguchi statistical design was applied and processed in the "Design expert 11 software". ANOVA was estimated using "t-test, F-value and P-value" were used to optimize the variables affecting the antimicrobial and antioxidant activities in both coral reef fungi. Significant differences between the eighteen designed media for each activity declared the importance of optimizing growth media variables before any experiment. The high agreement between the actual and predicted values indicated the accuracy of experimental work. The F-value and P-value declared the significance of the source models in both activities by the two coral fungi, along with that the salinity was the only influential variable in producing maximum antimicrobial and antioxidant activities by the two coral fungi.The regression analysis of the model revealed law standard deviation indicating the accuracy of the designed source model. The predicted R2, adjusted R2 and adequate precision values detected an adequate signal in software system.

In case of the coral reef fungal speciesA. ocharceopetaliformis, the optimum medium deduced from the system improved the antimicrobial activity by 50% and improved the antioxidant activity by 75% compared to unoptimized media. In case of B.spectabilis, the optimized media improved the two activities by 50% each.

Keywords : Coral fungi, Endosymbionts, Antimicrobial activity, Antioxidant activity, Variables optimization, Taguchi design.

Introduction

The role of fungi in marine environment is remain extremely limited (Amend et al.2012). About 1.5 million species of the fungal kingdom has been reported but less than 10 % of them detected to date (Hawksworth 2001). The coral phylum Cnidaria included about 10,000 species (Zhang 2011) has been the most vastly studied with respect to dispersal of fungi.Amend et al.(2012) reported the diversity of fungal community associated with the coral Acroporahyacinthus , where the fungal community was correlated with the coral host rather than with the environmental differences.

Recently more hypotheses describe the fungal species associated with corals as potentially mutualistic either protect the holobiont from disease and infection (Rohwer et al.2002 , Reshef et al.2006 , Shnit Orland and Kushmaro 2009) or by recycling nitrogen molecules for the Symbiodinium uptake (Wegley et al.2007). Fungi associated with the coral may pass a continuum from commensalist to mutualist to parasite according to environmental

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conditions and the health of the coral (Golubic et al.2005 , Wegley et al.2007 , Lesser et al.2007 a , Thurber et al.2009).

In the recent years, marine microorganisms specially fungi have got more attention as a result of their capabilities to produce biologically active secondary metabolites (Jha & Xu 2004, Zhou et al. 2016, Kobayashi 2016). Wang et al.(2017) discovered bioactive compounds which have antimicrobial activity against many pathogens, isolated from fungal species Aspergillustritici SP2-8-1 which isolated from the coral Galaxeafascicularis. Putri et al.(2015) isolated 15 fungal isolates from soft coral Sinularia sp. which collected from Panjang Island of the North Java Sea and screened their antimicrobial activity against pathogenic fungi Candidaalbicans and Aspergillusflavus where the results detected that these fungal symbiont was able to inhibit the pathogenic fungal growth.Abd El-Hady et al.(2014) isolated three fungi (FC1 , FC2 and FC3) from the soft corals Sinularia sp. and Lobophyton sp. then their antioxidant activity were assayed and found that the mycelial and supernatant extracts from the static culture of the identified fungus FC2 "Emericella Unguis" and the unidentified fungus FC1 isolated from Sinularia sp. exhibited the highest free radical scavenging activity (FRSA) against DPPH reagent. The polysaccharide AVP which extracted from coral associated fungus Aspergillusversiocolor LCJ-5-4 showed antioxidant activity assayed by the scavenging abilities on DPPH (hydroxyl and superoxide radicals) (Chen et al.2012).

Techniques of statistical experimental design are very useful for an empirical optimization of nutritional and physical conditions such as carbon, nitrogen, phosphorus, pH, temperature and aeration. This techniques help in understanding the variables interactions and estimating the optimum concentration of each variable in the growth medium (Bhima et al. 2012).

In Taguchi system the achieved experimental true data were applied in the software of the system where analysis of variance (ANOVA) and co-efficient significance were estimated (t-test, F-value and P-value). This system supply efficient results and consistent interaction between variable (Roy, 1999). Taguchi system was used in several processes of optimization to improve production of biological compounds or to optimize variables in biological activities (Selim et al.2015).

Materials and Methods

In our previous study (Abd El-Rahman et al.2020), two endosymbiotic fungal species (Aspergillusochraceopetasliformis&Byssochlamysspectabilis) were isolated from corals collected from Ain El-Sokhna, Red Sea, Egypt.

The process of optimization using Taguchi orthogonal array divided into four phases (design , performing , analysis and validation). The design of Taguchi entails the establishment of different experimental variables showed as orthogonal array (OAs) to decrease errors of experiments and to increase reproducibility of the laboratory experiments (Prasad et al. 2005). In Taguchi's method , efficiency is measured by the variation of a parameter from its target value and a loss function [L(y)] is developed for the variations as represented by : $L(y) = K(y-m)2$. Where "K" represent the proportionality

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constant , "m" denotes the target value and "y" is the value of the yield obtained for each trail. The first step is the design phase, where eight variables (agitation , glucose concentration , peptone concentration , KH2PO4 concentration , MgSO4.7H2O concentration , rose Bengal concentration , temperature and salinity (ratio of sea water to distilled water) Table (1).

Table 1. Selected Fermentation Variables for Optimization Process and their Assigned Levels

Factors	Levels 1	Levels 2	Levels 3
A) Agitation	Static	Agitated (120 rpm)	------
B) Glucose (g/l)	5	10	20
C) Peptone (g/l)	2.5	5	10
D) KH2PO4 (g/l)	0.5	1	2
E) MgSO4.7H2O (g/l)	0.25	0.5	1
F) Rose bengal (g/l)	0.0165	0.033	0.066
G) Temperature (oc)	25	30	35
H) Salinity	(1 : 0)*	(1 : 1)**	(0 : 1)***

(*) : Sea water only, (**) : Equal volumes of distilled water and sea water,
(***) : Distilled water only.

The design of Taguchi provides different standard OAs and their corresponding linear graphs for optimization processes. The standard orthogonal array selected was L-18 (21 × 37) to examine eight variables contain one variable with two levels and seven factors with three levels. The size of experiment and the number of runs (18 experimental trails) represented by the symbolic array L-18 of experimental matrix with layout (21 × 37) array and assigned with (21) two levels , but the remaining factors were represented by three levels (37). The two levels of the first factor were coded as 1 and 2 , while the three levels of remaining factors were coded as 1 , 2 and 3 (Table 1). Each column in the OA design consisted of a number of conditions depending on the designed levels to each variable. The runs involved setting a combination of variable levels, and the variables diversity was studied by crossing the factors (Table 2). The whole experiment was performed in a triplicate manner (Prasad et al.2005 , Chang et al.2006).

Experiments of fermentation were performed in 250 ml Erlenmeyer flasks containing 100 ml of RB broth (production medium) by using inoculums of coral fungi (A.ochraceopetaliformis and B.spectabilis) maintained and freshly cultivated on RBA media. After the incubation time (11 days) , the mycelium was separated out by filtration. The culture supernatant then extracted with ethyl acetate (3 × 35 ml). The resulted organic phase was dried under vacuum using a rotary evaporator to give a solid or oily extract. From the 18 experimental trails each crude fungal extract was assayed for its antimicrobial and antioxidant activity. The results of experiments obtained were fitted in

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Taguchi software (Design-Expert 11) to analyze the interactive factors and individual influences. ANOVA was estimated to assess the culture optimum conditions and to determine the effect of each selected variable in the activity of the antimicrobial and the antioxidant activities of the coral endosymbiotic fungi A.ochraceopetaliformis and B.spectabilis.

Table 2. Fractional factorial design of L-18 (21 × 37) orthogonal array that was used for process of fermentation with 18 probabilities from the 3 levels interactions of eight different factors resulted in 18 different media with the following compositions

Medium Number	Designed Experiments / Levels of different Factors							
	Agitation (rpm)	Glucose (g/l)	Peptone (g/l)	KH2PO4 (g/l)	MgSO4.7H2O (g/l)	Rose Bengal (g/l))	Temp. (OC)	Salinity Sw: Dw (ml)
1	Static	5	2.5	0.5	0.25	0.0165	25	1 : 0
2	Static	5	5	1	0.5	0.033	30	1 : 1
3	Static	5	10	2	1	0.066	35	0 : 1
4	Static	10	2.5	0.5	0.5	0.033	35	0 : 1
5	Static	10	5	1	1	0.066	25	1 : 0
6	Static	10	10	2	0.25	0.0165	30	1 : 1
7	Static	20	2.5	1	0.25	0.066	30	0 : 1
8	Static	20	5	2	0.5	0.0165	35	1 : 0
9	Static	20	10	0.5	1	0.033	25	1 : 1
10	Agitated	5	10	2	1	0.033	30	1 : 0
11	Agitated	5	2.5	0.5	0.25	0.066	35	1 : 1
12	Agitated	5	5	1	0.5	0.0165	25	0 : 1
13	Agitated	10	2.5	1	1	0.0165	35	1 : 1
14	Agitated	10	5	2	0.25	0.033	25	0 : 1
15	Agitated	10	10	0.5	0.5	0.066	30	1 : 0
16	Agitated	20	2.5	2	0.5	0.066	25	1 : 1
17	Agitated	20	5	0.5	1	0.0165	30	0 : 1
18	Agitated	20	10	1	0.25	0.033	35	1 : 0

Sw : Sea water , Dw : Distilled water

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Assay of the Antioxidant Activity of Coral Associated Fungi Using Free Radical Scavenging (FRS) Model

The assay was performed according to (Hamed 2009). One mg of EtOAc extract of each of the fungal species were dissolved in 1 ml DMSO to prepare a stock solution of 1000 ug/ml. 0.0035 g DPPH was dissolved in 100 ml of methanol HPLC grade to prepare 0.0035 % solution and stored in dark bottle until use. 0.1 ml of stock solution was added to 0.9 ml of methanolic DPPH solution , as the maximum concentration of tested samples 100 ug/ml. Then the reaction mixture was incubated for 30 mins , after that the reaction mixture was measured at wave length 540 nm by (JENWAY 6300) spectrophotometer. The blank was prepared by replacing 0.1 ml of samples by 0.1 ml of dissolving agent (DMSO). All assays were run in triplicates. The free radicals scavenging activity of symbiotic fungal extracts was calculated by the following equation :

$$\% \text{ Scavenging activity} = \{ (A_{blank} - A_{sample}) / A_{blank} \} \times 100$$

where , A_{blank} (Absorbance of reaction mixture without test sample DPPH + DMSO)
A_{sample} (Absorbance of reaction mixture in presence of test samples).

Assay of the Antimicrobial Activity of Coral Associated Fungi

The assay was carried out by agar disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI). Paper discs were impregnated with 20 mg of the extracts dissolved in 1 ml DMSO. Then discs were placed upon agar plates inoculated with bacterial or fungal pathogens and incubated at 37oc for 24 hr. At the end of the incubation time, the inhibition zone diameters (mm) around discs were measured. Negative controls were only treated with DMSO. Relative activity was calculated in response to the standards antibiotics used.

Experimental Results

Optimization of antioxidant activity of coral endosymbionts Aspergillusochraceopetasliformis&Byssochlamysspectabilis:

Table 3. Diagnostic case statistical study (t-test) of the actual and predicted values of antioxidant activity of A.ochraceopetaliformis&B.spectabilis

Exp Media	Actual value (%)		Predicted value (%)		Residual value (%)		t-test	
	A*	B**	A*	B**	A*	B**	A*	B**
1	10.40	3.90	11.30	8.18	-0.900	-4.28	0.0820	-0.676
2	16.40	9.10	15.72	11.90	0.6833	-2.80	0.062	-0.442
3	26.10	26.40	37.30	21.17	-11.2	5.23	-1.018	0.825
4	12.80	29.00	37.30	21.17	-24.50	7.83	2.227-	1.235
5	10.70	6.60	11.30	8.18	-0.6000	-1.58	-0.055	-0.250

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6	17.20	5.30	15.72	11.90	1.48	-6.60	0.167	-1.041
7	22.70	1.60	37.30	21.17	-14.60	-19.57	-1.327	-3.086
8	8.10	12.20	11.30	8.18	-3.20	4.02	-0.291	0.633
9	13.80	9.40	15.72	11.90	-1.92	-2.50	-0.174	-0.394
10	14.80	11.10	11.30	8.18	3.50	2.92	0.318	0.460
11	14.50	20.20	15.72	11.90	-1.22	8.30	-0.111	1.309
12	54.40	27.80	37.30	21.17	17.10	6.63	1.555	1.046
13	19.30	16.70	15.72	11.90	3.58	4.80	0.326	0.757
14	67.00	19.80	57.30	21.17	9.70	-1.37	0.881	-0.216
15	11.40	9.60	11.30	8.18	0.1000	1.42	0.009	0.223
16	13.10	10.70	15.72	11.90	-2.62	-1.20	-0.238	-0.189
17	40.80	22.40	37.30	21.17	3.50	1.23	0.318	0.195
18	12.40	5.70	11.30	8.18	1.10	-2.48	0.100	-0.392

(A*) : A.ochraceopetaliformis, (B**) :B.spectabilis.

The data in table (3) revealed that there were high agreement between the actual experimental values with the predicted values in both fungal species which indicated by the statistical case study (t-test) in the Taguchi system.

In case of A.ochraceopetaliformis the antioxidant actual values varied significantly within media variation which ranged between 8.10 % to 67.00 % of free radicals scavenging activity. In case of B.spectabilis the variation in antioxidant activity ranged between 1.60 % and 29.00 %. These variations reported the importance of media optimization before any biological experiments.

Similarly, the predicted values of antioxidant activity varied between 11.30 % to 57.30 % allover the 18 media used in case of A.ochraceopetaliformis and between 8.18 % to 21.17 % in case of B.spectabilis. Antioxidant activity in A.ochraceopetaliformis showed maximum value in medium (14) with residual value from the predicted value of 9.70 %. However minimum value had been reported in medium (8) with residual value of -3.20 %. Whereas, maximum antioxidant activity in B.spectabilis was achieved in medium (4) and minimum value was attained in medium (7).

The validation of the model was revealed based on the plot of normal probability of the residual values versus predicted one of the antioxidant activity. The plot showing straight line indicating that there is no abnormalities in the residuals (figure 1).

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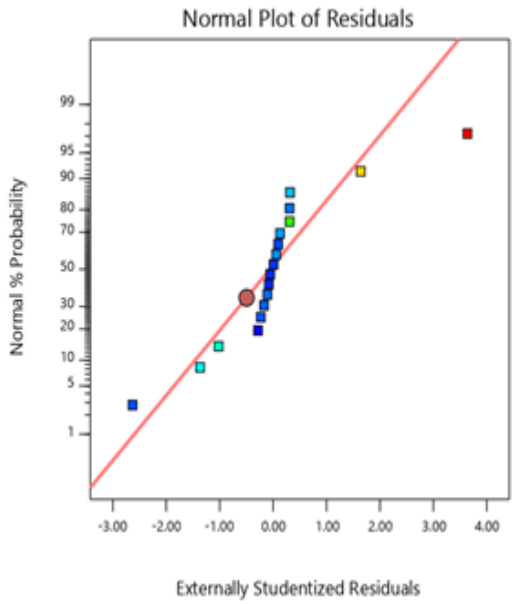

Figure 1.a A.ochraceopetaliformis

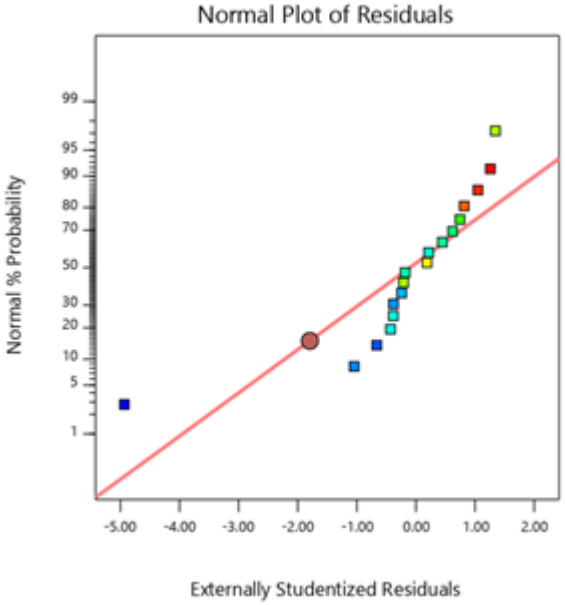

Figure 2.b B.spectabilis

Figure (1- a&b) : Normal probability plot of the studentized residuals to check the normality of residuals.

In case of the coral endosymbionts the plot of residual values versus predicted one of the antioxidant activity checked the constant error (figure 2). The residual values spread vertically were the same across all levels of the predicted values which detected that the residual value size was independent of its predicted values and validating the model.

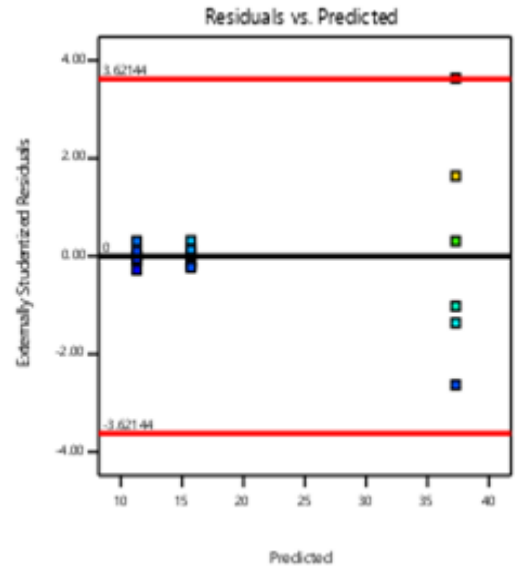

Figure 2.a A.ochraceopetaliformis

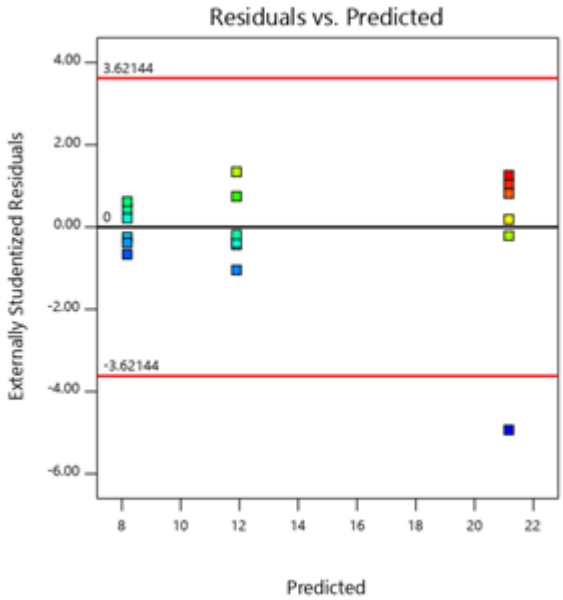

Figure2.b B.spectabilis

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Figure (2- a&b) : Studentized residuals versus predicted response values.

Table 4. ANOVA estimation in the model variablesfor antioxidant activity of A.ochraceopetaliformis strain CBS123.55 &B.spectabilis strain PAEC :

Source	**Sum of Squares**		**Mean Square**		**F-value**		**P-value**		**Model**	
	A*	B**	A*	B**	A*	B**	A*	B**	A*	B**
Model	2322.69	536.50	1161.35	268.25	8.00	5.56	0.0043	0.0156	Significant	Significant
Salinity	2322.69	536.50	1161.35	268.25	8.00	5.56	0.0043	0.0156		
Residual	2177.99	723.68	145.20	48.25						
Cor Total	4500.68	1260.18								

ANOVA reported that the F-value of the model is equal 8.00 and 5.56 in case of A.ochraceopetaliformis and B.spectabilis, respectively (table 4). This mean that the models are significant as the level of F-value less than 4 means insignificant model in the statistical system. In Taguchi design, P-value less than 0.05 indicated significant variable in the antioxidant activity. In this experiment salinity is the only variable significant with P-value 0.0043 and 0.0156 in A.ochraceopetaliformis and B.spectabilis, respectively, the other variables are insignificant.

Table 5. Regression analysis of significant variable in antioxidant activity byA.ochraceopetaliformis CBS123.55 and B.spectabilis PAEC:

Variable	**Target**	**St.dev.**		**R2**		**Adjusted R2**		**Predicted R2**		**Adequate Precession**	
		A*	**B****	**A***	**B****	**A***	**B****	**A***	**B****	**A***	**B****
Salinity	Antioxidant Activity	0.05	0.095	0.5161	0.4257	0.4516	0.3492	0.3031	0.1731	5.2853	4.5786

The regression analysis (table 5) of the significant variable (Salinity) showed low standard deviation 0.05 and 0.095 in case of A.ochraceopetaliformis and B.spectabilis, respectively for the antioxidant activity which means an accuracy of the designed models. The value of predicted R2 equal 0.3031 and 0.1731are in reasonable agreement with the adjusted R2 of 0.4516 and 0.3492, i.e the difference is less than 0.2. The adequate precision values of 5.2853 and 4.5786 detect an adequate signal in software system where the value greater than 4 is accepted in the system.

The optimal variables in medium of the coralA.ochraceopetaliformis deduced from the design system for maximum antioxidant activity was :

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Agitation : 200 rpm, Glucose : 10 g/l, Peptone : 5 g/l, KH2PO4 : 2 g/l, MgSO4.7H2O : 0.25 g/l., Rose Bengal : 0.033 g/l, Temp. : 25oc and Salinity (SW : DW) : 0:1 .

Where as the optimal variables in medium of the coral B.spectabilisdeduced from the design system for maximum antioxidant activity was :

Agitation : Static, Glucose : 10 g/l, Peptone : 2.5 g/l, KH2PO4 : 0.5 g/l, MgSO4.7H2O : 0.5 g/l, Rose Bengal : 0.033 g/l, Temp. : 35oc and Salinity (SW : DW) : 0:1 .

The salinity factor reported as the only significant factor in antioxidant activity of the coral endosymbionts A.ochraceopetaliformis strain CBS123.55 and B.spectabilis strain PAEC.

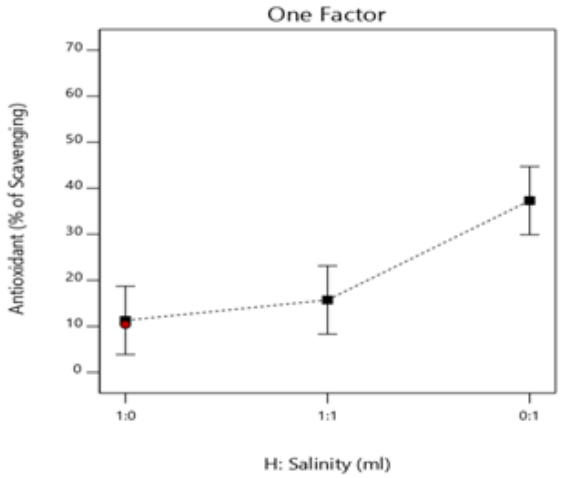

Figure 3.a A.ochraceopetaliformis

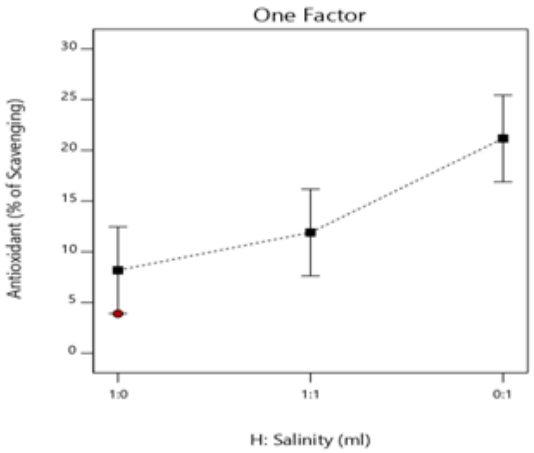

Figure 3.b B.spectabilis

Figure (3-a&b) : The influence of Salinity factor on the antioxidant activity.

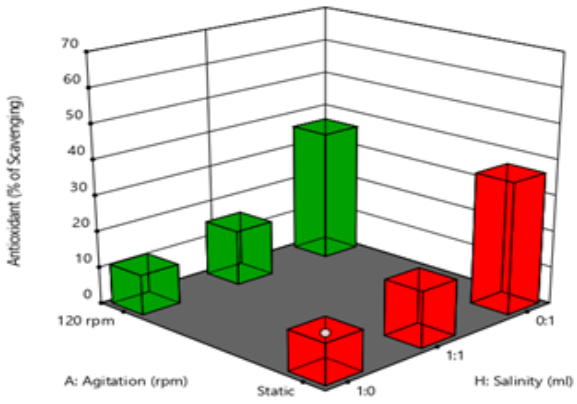

Figure 4.a A.ochraceopetaliformis

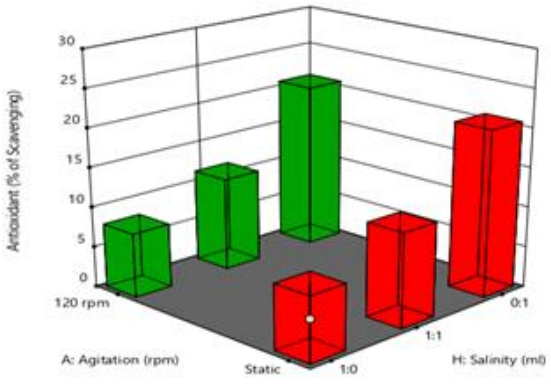

Figure 4.b B.spectabilis

Figure (4-a&b) : Interaction between salinity and agitation factor.

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Optimization of antimicrobial activity of coral endosymbionts Aspergillusochraceopetasliformis&Byssochlamysspectabilis :

It was found from the true experimental data that on RBA medium A.ochraceopetaliformis strain CBS123.55 and B.spectabilis strain PACE reported low activity against the bacterial pathogen E.coli ATCC11775. Consequently, media optimization were carried out to improve their antimicrobial activities.

Table 6. Diagnostic case statistical study (t-test) of the actual and predicted antimicrobial values of A.ochraceopetaliformis&B.spectabilis against E.coli :

Exp Media	Actual value		Predicted value		Residual value		t-test	
	A*	B**	A*	B**	A*	B**	A*	B**
1	11.00	12.00	9.50	10.17	1.50	1.83	0.545	1.195
2	9.00	11.00	9.67	9.50	-0.6667	1.50	-0.242	0.978
3	15.00	15.00	15.67	15.00	-0.6667	0.00	-0.242	0.00
4	19.00	14.00	15.67	15.00	3.33	-1.00	1.212	0.652-
5	9.00	10.00	9.50	10.17	-0.50	-0.1667	-0.182	-0.109
6	9.00	7.00	9.67	9.50	-0.6667	-2.50	-0.242	-1.630
7	17.00	13.00	15.67	15.00	1.33	-2.00	0.485	-1.304
8	8.00	10.00	9.50	10.17	-1.50	-0.1667	-0.545	-0.109
9	12.00	10.00	9.67	9.50	2.33	0.500	0.848	0.326
10	10.00	9.00	9.50	10.17	0.50	-1.17	0.182	-0.761
11	7.00	10.00	9.67	9.50	-2.67	0.500	-0.970	0.326
12	12.00	19.00	15.67	15.00	-3.67	4.00	-1.333	2.608
13	10.00	11.00	9.67	9.50	0.3333	1.50	0.121	0.978
14	22.00	15.00	15.67	15.00	6.33	0.00	2.303	0.00
15	11.00	9.00	9.50	10.17	1.50	-1.17	0.545	-0.761
16	11.00	8.00	9.67	9.50	1.33	-1.50	0.485	-0.978
17	9.00	14.00	15.67	15.00	-6.67	-1.00	-2.424	-0.652
18	8.00	11.00	9.50	10.17	-1.50	0.833	-0.545	0.543

The statistical case study (t-test) indicated that the actual values of the experimental 18 media were in close agreement with the predicted values in the software system except media 14 & 17 in case of A.ochraceopetaliformis and medium 12 in case of B.spectabilis as the values exceed the permissible limit (2.0) reported by the system (table 6).

It was revealed from the data that the actual experimental values of antimicrobial activity of A.ochraceopetalifromis strain CBS123.55 against E.coli ATCC11775 varied significantly among media in range between 7.00 to 22.00 mm and from 7.00 to 19.00 mm in B.spectabilis. This variability in the values of antimicrobial activity showed the importance of media optimization before any experiment. The values of the predicted antimicrobial activity also varied between 9.50 to 15.67 mm and from 9.50 to 15.00 inhibition zone diameter in A.ochraceopetaliformis and B.spectabilis, respectively.

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In A.ochraceopetaliformis the minimum actual antimicrobial activity was displayed in medium (11) with value of 7.00 mm inhibition zone diameter, whereas maximum antimicrobial value had been reported in medium (14) with actual value of 22.00, while in B.spectabilis the minimum and maximum actual values were recorded in media (6) and (12) with values of 7.00 and 19.00 mm inhibition zone diameter, respectively.

The designed model validation was measured based on the normal probability plot of the antimicrobial activity. The plot of normal probability of the residual values versus predicted values showing straight line indicated no abnormalities in the residuals as shown in figure (5- a&b).

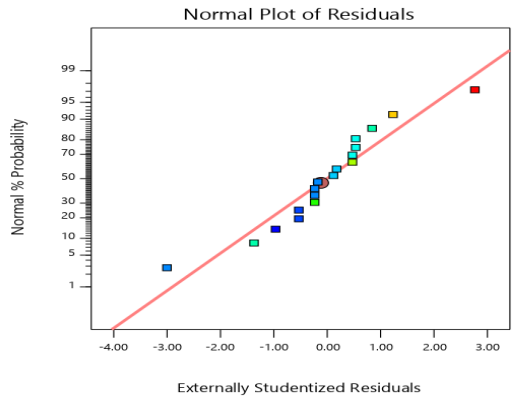

Figure 5.a A.ochraceopetaliformis

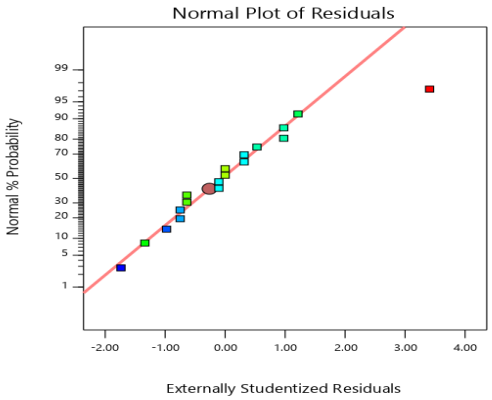

Figure5.b B.spectabilis

Figure (5-a&b) : Normal probability plot of the studentized residuals to check the normality of residuals.

The vertical spread of the residual values were the same across all levels of the predicted values of the antimicrobial activity which detected that the size of residual values was independent of its predicted values and validating the model (figure 6-a&b).

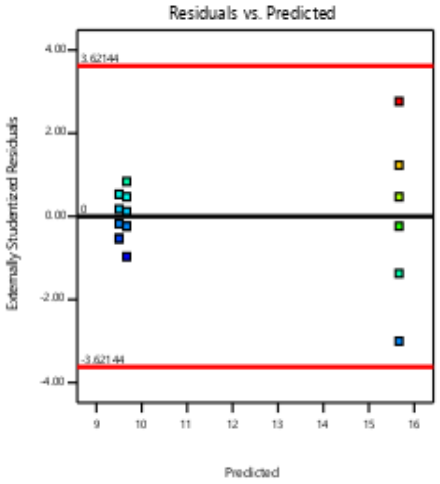

Figure 6.a A.ochraceopetaliformis

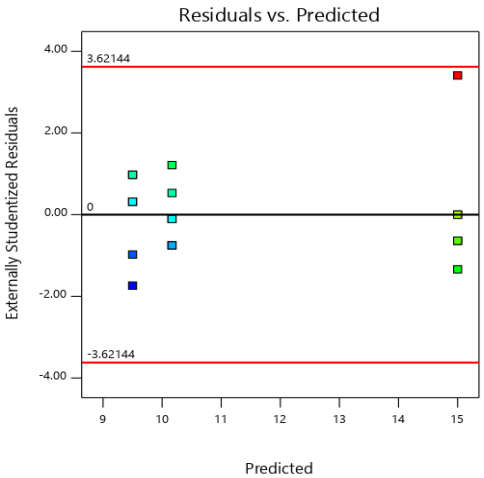

Figure 6.b B.spectabilis

Fig (6-a&b) : Studentized residuals versus predicted response values.

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Table 7. ANOVA estimation for the variable model in antimicrobial assay of A.ochraceopetaliformis CBS123.55 and B.spectabilis PAEC against E.coli

Source	Sum of Squares		Mean Square		F-value		P-value		Model	
	A*	B**	A*	B**	A*	B**	A*	B**	A*	B**
Model	148.11	108.11	74.06	54.06	8.16	19.15	0.0040	<0.0001	Significant	Significant
Salinity	148.11	108.11	74.06	54.06	8.16	19.15	0.0040	<0.0001		
Residual	136.17	42.33	9.08	2.82						
Cor Total	284.28	150.44								

ANOVA estimation of variance (table 7) revealed that the F-value of model is equal 8.16 and 19.15 for antimicrobial activity of A.ochraceopetaliformisand B.spectabilis, respectively against E.coli.This means that the modelsare significant as F-value less than 4 means insignificant model in the statistical system.

In Taguchi statistical system P-value less than 0.05 detected influencing variable in the process. In this experiment,salinity proved to be the only variable influencing in the production of secondary metabolites by A.ochraceopetaliformisand B.spectabilis with P-value of 0.004 and <0.0001, respectively.

Table 8. Regression analysis of the influencing variable in antimicrobial assay by coral fungal species A.ochraceopetaliformis and B.spectabilis against E.coli

Variable	Target	St.dev.		R2		Adjusted R2		Predicted R2		Adequate Precession	
		A*	B**	A*	B**	A*	B**	A*	B**	A*	B**
Salinity	Antimicrobial Activity	3.01	1.68	0.5210	0.7186	0.4571	0.6811	0.3103	0.5948	5.0134	8.0194

The regression analysis (table 8) of the variable (salinity) indicated that the predicted value (R2) 0.3103 and 0.5948 were in high agreement with the adjusted values (R2) 0.4571 and 0.6811 where the difference less than 0.2 according to system deduced high accuracy of experiment in A.ochraceopetaliformis and B.spectabilis, respectively. The adequate precision value of 5.0134 and 8.0194 detected an adequate signal in software system for antimicrobial activity of A.ochraceopetaliformis strain CBS123.55 and B.spectabilis PAEC against E.coli, where value of adequate precision greater than 4 is accepted in Taguchi statistical design.

The optimal variables in medium of the coral A.ochraceopetaliformis deduced from the design system was :

Agitation : 200 rpm, Glucose : 10 g/l, Peptone : 5 g/l, KH2Po4 : 2 g/l, MgSo4.7H2O : 0.25 g/l, Rose Bengal : 0.033 g/l, Temp. : 25oc and Salinity (SW : DW) : 0:1.

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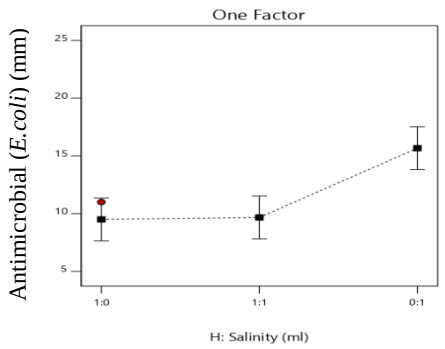
Whereas the optimal variables in medium of the coral B.spectabilis deduced from the design system was :

Agitation : 200 rpm, Glucose : 5 g/l, Peptone : 5 g/l, KH2Po4 : 1 g/l, MgSO4.7H2O : 0.5 g/l, Rose bengal : 0.0165 g/l, Temp. : 25oc and Salinity (SW : DW) : 0:1.

The significant influential factor in antimicrobial activity of A.ochraceopetaliformis and B.spectabilis against E.coli was Salinity only.



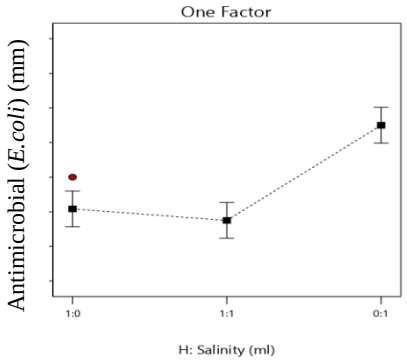
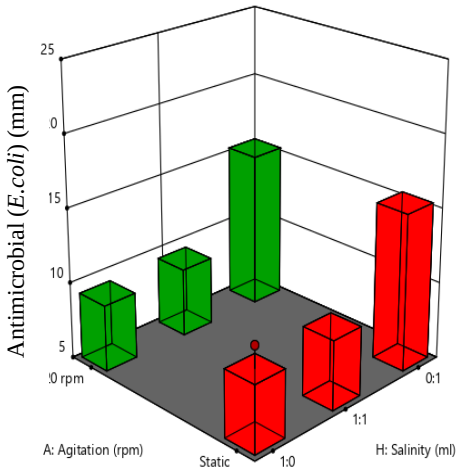


Figure 7.a A.ochraceopetaliformis **Figure7.b** B.spectabilis

Figure (7-a&b) : The influence of Salinity variable on the antimicrobial activity.



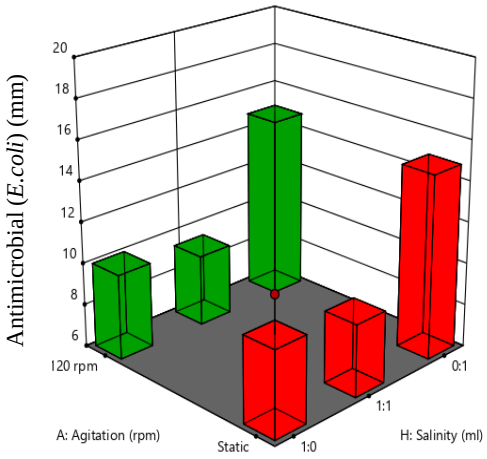


Figure 8.a A.ochraceopetaliformis **Figure8.b** B.spectabilis

Figure (8-a&b) : Interaction between salinity with agitation variables.

Discussion

Experimental statistical design system is crucial to optimize the nutritional and environmental conditions during cultivation where it statistically help in understanding the

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variables interaction and to determine the significant factors affecting the process and the optimum concentration of all factors.

In the present study, the system of Taguchi design was applied and the experimental data was processed in the "Design Expert 11" software. Analysis of variance (ANOVA) was estimated using t-test, F-value and P-value which result in high degree of consistency and a reproducibility rarely found in other statisticaldesign systems(Roy,1999). Antimicrobial and antioxidant activities of the coral endosymbiotic fungal species A.ochraceopetaliformis and B.spectabilis against E.coli ATCC11775, indicated that the actual experimental values and predicted values showed high difference among the 18 media designed. This phenomenon displayed the importance of the optimization process before experiments. The t-test indicated high agreement between the actual and predicted values in the Taguchi software system in antimicrobial and antioxidant experiments which revealed an accuracy of the experimental work.

In ANOVA estimation in both activities, F-values indicated that the source models are significant. Taguchi system analyses of P-values reported that the only influential variable in both activities of the two fungal strains was salinity.

Table 9. The optimal media deduced from the Taguchi system

Activity	**Optimum factors**							
	Agitation* (rpm)	**Glucose* (g/l)**	**Peptone* (g/l)**	**KH2PO4* (g/l)**	**MgSO4. 7H2O* (g/l)**	**Rose Bengal* (g/l)**	**Temp.* (oC)**	**Salinity (Sw:Dw)**
Antimicrobial (A.ochraceopetaliformis)	200	10	5	2	0.25	0.033	25	0:1
Antimicrobial (B.spectabilis)	200	5	5	1	0.5	0.0165	25	0:1
Antioxidant (A.ochraceopetaliformis)	200	10	5	2	0.25	0.033	25	0:1
Antioxidant (B.spectabilis)	Static	10	2.5	0.5	0.5	0.033	35	0:1

(*) Optimum factors which varied between 2 fungal strains.
(Sw) : Sea water, (Dw) : Distilled water.

The obtained optimal conditions improved the antimicrobial and antioxidant activities of A.ochraceopetaliformis by about 50 % and 75 %, respectively. While in B.sepctabilis the improvement of antimicrobial and antioxidant activities were about 50 % for both. It is noted from these results that low salinity [(0) Sw : (1) Dw] represented a stress factor and unfavorable environmental condition to marine coral fungi that enhance the induction of biologically active secondary metabolites A.ochraceopetalifromis and B.spectabilis. The stress environmental conditions may force these endosymbionts to produce secondary

metabolites, in invitro study which is the goal of search. Similarly, Bose et al.(2015) optimized the salinity and period of incubation for the growth of the marine actinobacterial species Salinisporaarenicola isolated from the Great Barrier Reef Sponge for maximum antibiotics production. They found that salt concentration have important effect in the production of antibiotics.

Taguchi system was also used in many optimization processes to enhance reproducibility or to optimize many biological activities. Selim et al.(2015), maximize antioxidant activity of the endophytic fungus Chaetomiumglobosum JN711454 during fermentation process by using design of Taguchi orthogonal array (OA) [with layout L18 (21 × 37)].The static condition with potato extract 25 g/100 ml represent the more favorable variables for the antioxidant production. As a result of the optimization experiment antioxidant activity improved to a level of two fold with 40 % enhancement in activity. Moreover,Ng et al. (2013)used Taguchi's L16 system in optimization of Streptomycesroseosporus NRRL11379 culture to increase daptomycin antibiotic production, where the maximum yield was isolated from the medium with 60 g/l dextrin, 10 g/l dextrose, 8 g/l yeast extract and 1 g/l molasses, respectively.

Hamed et al.(2016), used Taguchi orthogonal array (OA) model to increase production of L-methioninase by Chaetomiumglobosum KXO24450, where the experiment of optimization was designed to determine the effect of six factors, five levels each. The obtained optimum medium improved the L-methioninase production to 2225 U/mg. To improve desulphurization activity of Alternaria sp. Cf1 (KF564051) which isolated from Mihaliccik region (Turkey) Taguchi system applied. The optimum values obtained from the optimization experiment showed 52 % increase in total sulphur removal (Aytar et al.2014). Subhash & Mohan (2014), used methodology of Taguchi to increase bio-diesel production by Aspergillusawamori (MTCC11639). The experiment was designed to determined the effect of eight factors (21 × 37) (L18 experimental matrix). The significant factors were found to be pH, glucose and incubation temperature. The obtained medium increase lipid productivity by 31 %.

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